



Association of Variants in RETN with Plasma Resistin Levels and Diabetes-Related Traits in the Framingham Offspring Study

Citation

Hivert, Marie-France, Alisa K. Manning, Jarred B. McAteer, Josée Dupuis, Caroline S. Fox, L. Adrienne Cupples, James B. Meigs, and Jose C. Florez. 2009. Association of variants in RETN with plasma resistin levels and diabetes-related traits in the Framingham offspring study. *Diabetes* 58, no. 3: 750-756.

Published Version

doi://10.2337/db08-1339

Permanent link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:4727657>

Terms of Use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA>

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. [Submit a story](#).

[Accessibility](#)

Association of Variants in *RETN* With Plasma Resistin Levels and Diabetes-Related Traits in the Framingham Offspring Study

Marie-France Hivert,^{1,2} Alisa K. Manning,³ Jarred B. McAteer,^{4,5} Josée Dupuis,³ Caroline S. Fox,^{6,7} L. Adrienne Cupples,³ James B. Meigs,^{1,2} and Jose C. Florez^{2,4,5}

OBJECTIVE—The *RETN* gene encodes the adipokine resistin. Associations of *RETN* with plasma resistin levels, type 2 diabetes, and related metabolic traits have been inconsistent. Using comprehensive linkage disequilibrium mapping, we genotyped tag single nucleotide polymorphisms (SNPs) in *RETN* and tested associations with plasma resistin levels, risk of diabetes, and glycemic traits.

RESEARCH DESIGN AND METHODS—We examined 2,531 Framingham Offspring Study participants for resistin levels, glycemic phenotypes, and incident diabetes over 28 years of follow-up. We genotyped 21 tag SNPs that capture common (minor allele frequency >0.05) or previously reported SNPs at $r^2 > 0.8$ across *RETN* and its flanking regions. We used sex- and age-adjusted linear mixed-effects models (with/without BMI adjustment) to test additive associations of SNPs with traits, adjusted Cox proportional hazards models accounting for relatedness for incident diabetes, and generated empirical *P* values (*P_e*) to control for type 1 error.

RESULTS—Four tag SNPs (rs1477341, rs4804765, rs1423096, and rs10401670) on the 3' side of *RETN* were strongly associated with resistin levels (all minor alleles associated with higher levels, *P_e* < 0.05 after multiple testing correction). rs10401670 was also associated with fasting plasma glucose (*P_e* = 0.02, BMI adjusted) and mean glucose over follow-up (*P_e* = 0.01; BMI adjusted). No significant association was observed for adiposity traits. On meta-analysis, the previously reported association of SNP -420C/G (rs1862513) with resistin levels remained significant (*P* = 0.0009) but with high heterogeneity across studies (*P* < 0.0001).

CONCLUSIONS—SNPs in the 3' region of *RETN* are associated with resistin levels, and one of them is also associated with glucose levels, although replication is needed. *Diabetes* 58: 750–756, 2009

Adipose tissue is now recognized as a prolific endocrine organ. In the past few years, several proteins, called adipokines, produced by adipose tissue have been discovered (1). In the process of unraveling the link between obesity and development of diabetes, various adipokines have been suspected to contribute to the pathogenesis of insulin resistance. Resistin is a 12.5-kDa polypeptide that belongs to the resistin-like molecule family of cysteine-rich proteins (2). In murine models, it is produced mainly by adipocytes, and it has been proposed to link obesity with diabetes (3). In humans, adipocytes seem to contribute to only a small fraction of the resistin production (4), and macrophages are considered the predominant source of circulating resistin (5,6). Adipose tissue of obese individuals is characterized by increased infiltration by macrophages (7), which have been proposed to contribute to the proinflammatory state that is characteristic of insulin resistance. Some population studies (8–10) have shown that resistin levels are indeed associated with metabolic risk factors and insulin resistance, suggesting that resistin may play an important role in the pathophysiology of diabetes.

The gene encoding resistin (*RETN*) is located on chromosome 19p13. Genetic variants in *RETN* have been examined by many groups, and it is estimated that up to 70% of the variation in circulating resistin levels can be explained by genetic factors (11). Several single nucleotide polymorphisms (SNPs) have been associated with resistin levels (9,12–14). However, associations between *RETN* and BMI or other measures of adiposity have shown very inconsistent results (13,15–21). Polymorphisms in *RETN* also have been associated with indexes of insulin resistance in some reports (21,22), but lack of replication and null associations (13,19) have raised questions regarding the robustness of these findings. Moreover, most of the analyses examining common variation in *RETN* and risk of type 2 diabetes have been negative (9,13,16,23–27), with nominal associations only emerging in subanalyses (28,29). The inconsistencies in those studies might be due to low power afforded by small samples or poor coverage of the gene and of its flanking sequences.

To address those limitations, we conducted fine mapping of *RETN* to test if any of the SNPs in or around the gene are associated with resistin levels, diabetes incidence, or glycemic and obesity traits in the Framingham Offspring Study, a large representative community sample. Our goal was to confirm or refute previous reports of association and possibly uncover novel SNP associations

From the ¹General Medicine Division, Massachusetts General Hospital, Boston, Massachusetts; the ²Department of Medicine, Harvard Medical School, Boston, Massachusetts; the ³Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts; the ⁴Center for Human Genetic Research and Diabetes Research Center (Diabetes Unit), Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts; the ⁵Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts; the ⁶Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; and the ⁷National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, Massachusetts.

Corresponding author: Jose C. Florez, jcflorez@partners.org.

Received 29 September 2008 and accepted 10 December 2008.

Published ahead of print at <http://diabetes.diabetesjournals.org> on 15 December 2008. DOI: 10.2337/db08-1339.

© 2009 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

using comprehensive tag SNP linkage disequilibrium (LD) mapping.

RESEARCH DESIGN AND METHODS

The Framingham Offspring Study is a large community-based prospective cohort study designed to investigate cardiovascular disease risk factors. This analysis includes 2,531 participants (including 285 pedigrees and 1,445 unrelated individuals) who were followed over 28 years on a periodic basis (from exam 1 [1971–74] up to exam 7 [1998–2001]). Each exam cycle included anthropometric measurements, a physical exam, and blood samples related to cardiovascular risk factors. The study was approved by the institutional review boards of Massachusetts General Hospital, Boston University, and the Massachusetts Institute of Technology; written informed consent, including consent for genetic analyses, was obtained from all study participants.

Participants underwent standardized procedures for all anthropometric measurements (weight, height, and waist circumference [at the umbilicus]). BMI was calculated using measured weight (kg) and the square of height (m^2). Diabetes was defined by 2003 American Diabetes Association clinical criteria, where case subjects were defined as those who used oral hypoglycemic or insulin therapy at any exam or had a fasting plasma glucose (FPG) ≥ 7.0 mmol/l at the index exam and FPG ≥ 7.0 mmol/l on at least one prior exam. Fasting resistin levels were measured once at exam 7. For diabetes, we were primarily interested in outcomes and metabolic traits measured over follow-up (time-averaged mean FPG over follow-up [exams 3–7, chosen for measurement stability]), and at the last follow-up (exam 7) including FPG; fasting insulin; homeostasis model assessment of insulin resistance (HOMA-IR) (30); A1C levels; the Gutt 0- to 120-min insulin sensitivity index (31), conducted in a subsample; BMI; waist circumference; visceral adipose tissue (VAT); and subcutaneous adipose tissue (SAT), measured by computerized tomography (the latter two conducted in a subsample) (32). FPG was measured immediately with a hexokinase reagent kit (A-gent glucose test; Abbott, South Pasadena, CA), and A1C was measured by high-performance liquid chromatography (33). Other plasma analyses were frozen at -80°C until assay: fasting plasma insulin was measured with a human-specific insulin assay (Linco, St. Louis, MO), and fasting total resistin levels were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Intra-assay coefficients of variation were $<3\%$ for glucose, 6.1% for insulin, and 9.0% for resistin.

SNP selection. We downloaded SNPs from the region of interest (20 kb on the 5' end plus 10 kb on the 3' end of *RETN*) from the phase 2 HapMap database (www.hapmap.org) in January 2006. Due to sparse coverage of the region, we then mined the dbSNP database to choose additional SNPs across the region so as to ensure adequate coverage. We genotyped a set of 58 SNPs in the HapMap European-descent CEU plate, and 27 of them passed quality-control metrics (nonmonomorphic in CEU, minor allele frequency [MAF] > 0.05 , and Hardy-Weinberg equilibrium [HWE] P value > 0.001). We used Tagger (www.broad.mit.edu/mpg/tagger) to select 21 tag SNPs using a pairwise approach to capture (with an $r^2 > 0.8$) the 27 SNPs that passed quality control in the region of interest. Previously reported SNPs were forced in the tagging approach.

Genotyping. Genotyping was performed on the iPLEX Sequenom platform. To correctly estimate MAF and HWE, we performed these calculations in a maximally unrelated subset of individuals. The genotyping success rate was $>95\%$ for all the LD-tagging SNPs (average 98.4%). Consensus rates on a subset of 254 duplicate individuals reached 99.6%. All the SNPs met HWE ($P > 0.001$).

Statistical analysis. The quantitative traits were regressed against covariates in order to produce Studentized residuals, which were used as the dependent variable in the subsequent genetic models. Two covariate adjustment schemes were used: the first with sex, age, and age² adjustment and the second with BMI added to age and sex to examine the strength of the SNP associations when adjusted for overall adiposity. For resistin levels and glucose-related traits (mean glucose exam 3–7, FPG, fasting insulin, HOMA-IR, A1C, and Gutt 0- to 120-min insulin sensitivity index), we excluded participants with diabetes.

The association between each trait residual and each SNP was assessed using a linear mixed-effects (LME) model implemented in SOLAR (34) to account for the within-family correlation of the trait. Each SNP was included in a model as a fixed effect with additive coding. The models included random effects to account for the covariance between family members; the covariance structure was determined by the degree of relatedness between each relative pair.

To assess SNP associations with type 2 diabetes, we used Cox proportional hazards survival analysis, with diabetes as the outcome and the survival time as the age at the exam at which diabetes was first determined. The survival time of individuals without diabetes was the age at their last exam. The model

was implemented with the survival package in R (35), with the same adjustments as in the LME models, with covariates taken at the first exam. Trait correlation among siblings was modeled with a frailty term in the survival model (36).

Statistical significance was determined using an empirical P value (P_e) obtained by a simulation strategy, which generated a null distribution of minimum P values. We simulated a trait for our sample using SIMQTL in SOLAR (34). The heritability of the trait was 35%, although similar null distributions were obtained for heritabilities of 15 and 50%. The simulated trait was analyzed in the same manner as the trait residuals, using LME models implemented in SOLAR, and the minimum P value observed over all the SNPs was recorded. A total of 10,000 traits were generated, producing an empirical distribution of minimum P values. This strategy provides multiple SNP-testing correction accounting for the correlation among the SNPs but does not correct for the multiple traits being tested.

Multiple SNP models. When we observed multiple SNP associations for the same trait, and the associated SNPs were in moderate to high LD, we considered the possibility that the association signals were not independent. To assess whether these association signals were simply due to LD among the SNPs, we sequentially added SNPs to the LME models: if the signals were independent, we expected that they would each remain significant in these models.

BMI interaction model. Based on a previous report (28), we assessed the effect of a BMI \times genotype interaction with SNP rs3745367 (also known as IVS2 + 181G/A) in the LME model by adding a first-level interaction term (BMI \times rs3745367) to predict diabetes incidence. Also, since the associations of rs10401670 with glucose seemed stronger in BMI-adjusted models, we explored the interaction of rs10401670 and BMI to predict diabetes incidence.

Meta-analysis of association between SNP -420C/G (rs1862513) and resistin levels. The variation in *RETN* most often reported in the previous literature is the promoter SNP -420C/G (rs1862513). We included rs1862513 in our tags to confirm or refute previous findings with resistin levels. Since many groups have reported associations with resistin levels, we decided to perform a meta-analysis of our results and published reports by requesting crude data from the corresponding authors. The meta-analyses of the relationship between rs1862513 with resistin levels were performed using the inverse-variance method for pooling regression coefficients with a random effects estimate based on the DerSimonian-Laird method (37).

RESULTS

Characteristics of the participants genotyped in the Framingham Offspring Study are presented in Table 1. Overall, 2,531 participants were included in this analysis, 53% were women, and 10% had a diagnosis of diabetes over the 28 years of follow-up. Mean resistin levels measured at exam 7 were 14.1 ± 7.2 ng/dl. The heritability of resistin levels in the Framingham Offspring Study was estimated to be 35% (adjusted for sex, age, age², and BMI). Other metabolic traits measured at exam 7 and the mean glucose levels over exams 3–7 are presented in Table 1. Resistin levels were modestly correlated with BMI ($r = 0.16$), waist circumference ($r = 0.18$), VAT ($r = 0.15$), and SAT ($r = 0.13$; all correlations age and sex adjusted; all P values < 0.001).

With 21 tag SNPs selected by a tagging approach that set an $r^2 > 0.8$, we were able to capture 96% (26 of 27 SNPs that passed quality control in the CEU plates) of SNPs in the region of interest at an $r^2 > 0.8$ and 100% at an $r^2 > 0.7$ (see supplementary Table 1 for details regarding coverage [available at <http://diabetes.diabetesjournals.org/cgi/content/full/db08-1339/DC1>]). Average distance between tag SNPs was 1.5 kb. The tag SNPs are shown in Table 2, with their location on chromosome 19 (NCBI B35 assembly), relation to *RETN* itself (in and around the gene), and other names given in prior publications. SNP rs3745368 was not followed further due to its low MAF (0.002) in our sample.

The LD map of the genotyped region is presented in supplementary Fig. 1 (D' statistics). *RETN* is a short gene spanning only 1,369 bp. A gene coding for an open reading frame (*C19orf59*) also known as mast cell-expressed membrane protein 1 (*MCEMP1*) is located downstream of

TABLE 1
Characteristics of 2,531 Framingham Offspring Study participants genotyped for *RETN* variants

	<i>n</i>	
Population demographic		
Unrelated	1,445	
Pedigrees	285	
Sibpairs	989	
Avuncular pairs	66	
Cousins	653	
Sex (% of female)	2,531	53
Exam 7 characteristics		
Age (years)	2,482	61 ± 9.6
BMI (kg/m ²)	2,394	28.2 ± 5.4
Waist circumference (inches)	2,377	39.3 ± 5.6
VAT (cm ³)	1,018	2,139 ± 1,100
SAT (cm ³)	1,018	2,983 ± 1,312
Fasting blood glucose (mg/dl)*	2,198	100.3 ± 18.0
Fasting insulin (μU/ml)*	2,158	14.5 ± 8.4
HOMA-IR*	2,158	3.7 ± 2.7
Gutt insulin sensitivity index*	815	21.7 ± 7.3
A1C (%)*	2,025	5.5 ± 0.7
Resistin levels (ng/dl)*	1,877	14.1 ± 7.2
Longitudinal follow-up		
Mean fasting glucose exams 3–7 (mg/dl)†	2,515	99.3 ± 20.4
Diabetes (%)‡	2,531	10

Data are means ± SD or percent, unless otherwise indicated. *After removing participants with diabetes. †Over exams 3–7 because of stability of measurements. ‡Over exams 1–7, 28 years of follow-up on average.

the 3' end of *RETN* and was fully captured by our tagging approach, with our last downstream SNP being on the 3' side of *MCEMP1*. The LD map for the tagging SNPs in the Framingham population is shown in supplementary Fig. 2.

Circulating resistin levels were measured in 1,877 geno-

TABLE 2
Characteristics of SNPs genotyped in and around *RETN* in 2,543 participants in the Framingham Offspring Study

SNP identification	Position (NCBI 35)	Relation to the <i>RETN</i> gene	Other name	Call rate	HWE <i>P</i>	Strand	Major allele	Minor allele	MAF
rs794070	7620814	5' of promoter		0.97	0.32	+	T	C	0.21
rs11883223	7628636	5' of promoter		0.99	0.11	–	G	A	0.17
rs2081075	7629461	5' of promoter		0.97	0.28	+	G	A	0.29
rs10418380	7630540	5' of promoter		0.95	0.76	+	A	G	0.32
rs10413807	7630628	5' of promoter		0.99	0.69	–	G	C	0.21
rs12460483	7636594	5' of promoter		1.00	0.71	–	G	A	0.12
rs12459044	7638406	Promoter		0.99	0.15	–	C	G	0.14
rs7408174	7638955	Promoter		0.97	0.54	–	T	C	0.31
rs1862513	7639793	Promoter	–420C/G	1.00	0.57	–	G	C	0.29
rs3219177	7640369	Intron 2	IVS2 + 39C/T	0.99	0.35	–	C	T	0.21
rs3745367	7640511	Intron 2	IVS2 + 181G/A	0.99	0.67	+	G	A	0.24
rs3219178	7640951	Intron 3	IVS3 + 167C/G	0.97	1.00	+	C	G	0.42
rs10402265	7641089	Intron 3		0.99	1.00	–	C	G	0.14
rs3745368	7641297	3' UTR	3' UTR + 62G/A	0.98	1.00	–	G	A	0.002
rs3745369	7641475	3' of 3' UTR		0.99	0.80	+	G	C	0.50
rs1477341	7642799	3' of 3' UTR		0.99	0.87	–	A	T	0.44
rs4804765	7643840	3' of 3' UTR		0.98	0.64	–	G	T	0.33
rs1423096	7645177	3' of 3' UTR		0.99	0.53	+	G	A	0.09
rs10401670	7648802	3' of 3' UTR*		0.98	0.75	–	C	T	0.43
rs11882592	7649975	3' of 3' UTR*		0.98	0.07	–	C	A	0.18
rs10411016	7651043	3' of 3' UTR		0.98	0.79	–	T	G	0.49

*Located in chromosome 19 open reading frame 59 (C19orf59), also known as mast cell-expressed membrane protein 1 (MCEMP1).

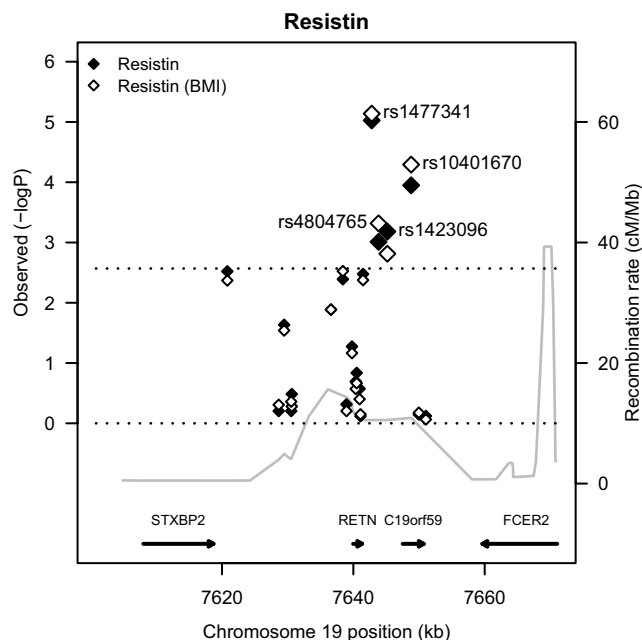


FIG. 1. Negative log base 10 of the *P* value for genetic associations for resistin levels under the additive model (left Y-axis), graphed versus SNPs in the *RETN* region arranged by chromosomal position (X-axis). The continuous line marked by the right Y-axis indicates the recombination rate. The *RETN* and *C19orf59* (also known as mast cell-expressed membrane protein 1 [*MCEMP1*]) genes are shown by the horizontal arrows at the bottom of the plot. ♦, traits adjusted for sex and age; ◇, additional adjustment for BMI.

typed participants without diabetes. The associations for each tag SNP with resistin levels is illustrated in Fig. 1. The mean resistin level for each genotype and the nominal (uncorrected) and empirical (corrected for the number of SNPs) *P* values for each tag SNP are presented in Table 3. We found that the minor alleles at four tag SNPs (rs1477341, rs4804765, rs1423096, and rs10401670) in the

TABLE 3

Mean circulating resistin levels per genotype at SNPs in or near *RETN* in the Framingham Offspring Study*

SNP identification	Total <i>N</i>	MAF (allele)	M/M		M/m		m/m		Nominal <i>P</i> [†]	Nominal <i>P</i> (BMI adjusted) [‡]		
			<i>n</i>	Means ± SD	<i>n</i>	Means ± SD	<i>n</i>	Mean ± SD		<i>P</i> _e [†]	<i>P</i> _e (BMI adjusted) [‡]	<i>P</i> _e (BMI adjusted) [‡]
rs794070	1,834	0.21 (C)	1,143	14.02 ± 6.59	612	14.91 ± 8.79	79	16.03 ± 9.96	0.003	0.051	0.004	0.07
rs11883223	1,852	0.17 (A)	1,294	14.36 ± 7.46	508	14.39 ± 7.33	50	14.41 ± 6.23	0.63	1.0	0.49	1.0
rs2081075	1,833	0.29 (A)	910	14.22 ± 7.79	765	14.35 ± 7.16	158	15.50 ± 6.93	0.02	0.31	0.03	0.36
rs10418380	1,773	0.32 (G)	830	14.26 ± 7.26	748	14.38 ± 7.39	195	14.19 ± 5.50	0.63	1.0	0.44	1.0
rs10413807	1,852	0.21 (C)	1,151	14.32 ± 6.63	622	14.33 ± 8.11	79	13.66 ± 5.17	0.33	1.0	0.52	1.0
rs12460483	1,875	0.12 (A)	1,446	14.23 ± 7.61	407	14.94 ± 7.32	22	14.15 ± 4.69	0.01	0.20	0.01	0.20
rs12459044	1,852	0.14 (G)	1,365	14.21 ± 7.61	468	14.96 ± 7.42	19	14.60 ± 5.58	0.004	0.07	0.003	0.051
rs7408174	1,827	0.31 (C)	901	14.32 ± 7.64	759	14.26 ± 6.88	167	14.40 ± 6.33	0.48	1.0	0.62	1.0
rs1862513	1,876	0.29 (C)	937	13.98 ± 6.47	786	14.86 ± 8.82	153	14.43 ± 5.90	0.05	0.57	0.07	0.66
rs3219177	1,866	0.21 (T)	1,141	14.31 ± 7.56	650	14.63 ± 7.75	75	13.85 ± 4.78	0.20	0.96	0.27	0.99
rs3745367	1,867	0.24 (A)	1,058	14.23 ± 7.28	700	14.71 ± 8.19	109	14.01 ± 5.07	0.15	0.90	0.22	0.97
rs3219178	1,826	0.42 (G)	603	14.67 ± 8.00	896	14.26 ± 7.48	327	13.96 ± 5.76	0.27	0.99	0.40	1.0
rs10402265	1,858	0.14 (G)	1,369	14.33 ± 7.35	451	14.61 ± 7.70	38	13.13 ± 4.17	0.76	1.0	0.71	1.0
rs3745369	1,861	0.50 (C)	456	13.67 ± 6.45	937	14.45 ± 7.73	468	15.01 ± 8.05	0.003	0.056	0.004	0.07
rs1477341	1,867	0.44 (T)	581	13.66 ± 7.16	908	14.48 ± 7.06	378	15.22 ± 8.34	9.4×10^{-6}	0.0005	7.3×10^{-6}	0.0005
rs4804765	1,836	0.33 (T)	831	14.03 ± 7.49	806	14.47 ± 6.90	199	15.26 ± 9.16	0.0010	0.02	0.0005	0.009
rs1423096	1,858	0.09 (A)	1,496	14.16 ± 7.50	338	15.12 ± 6.65	24	16.50 ± 9.90	0.0007	0.01	0.0015	0.03
rs10401670	1,852	0.43 (T)	596	13.91 ± 7.79	909	14.37 ± 6.97	347	15.19 ± 8.40	0.0001	0.004	5.1×10^{-5}	0.002
rs11882592	1,843	0.18 (A)	1,273	14.39 ± 7.51	529	14.25 ± 6.87	41	16.66 ± 14.03	0.71	1.0	0.67	1.0
rs10411016	1,843	0.49 (G)	475	14.64 ± 8.53	929	14.16 ± 6.56	439	14.48 ± 7.73	0.76	1.0	0.86	1.0

*Resistin levels in ng/dl. †Adjustment for sex, age, and age². ‡Adjustment for sex, age, age², and BMI. M, major allele; m, minor allele.

3' region of *RETN* were associated with higher resistin levels (all $P_e < 0.05$). Since some of those SNPs were in moderate LD in the Framingham cohort (see supplementary Table 6 for specific D' and r^2 values), we conducted multiple SNPs models. When models were examined with various combinations of these SNPs, rs4804765 and rs1423096 had independent associations with resistin levels and rs4804765 explained the association of the two other SNPs (rs1477341 and rs10401670). The best-fitting model included rs4804765 and rs1423096 and explained 1.5% of the variance in resistin levels.

One of these SNPs in the 3' region, rs10401670, was also associated with mean glucose over follow-up ($P_e = 0.02$, after BMI adjustment $P_e = 0.01$) and FPG at exam 7 ($P_e = 0.10$, after BMI adjustment $P_e = 0.02$): its minor T allele was associated with higher glucose levels, concordant with a potential effect of its association with higher resistin levels. Two other SNPs showed associations with FPG at exam 7 (rs1423096, $P_e = 0.049$; and rs10413807, $P_e = 0.02$) but did not remain significant after adjustment for BMI. No other associations were observed in the glycemic or adiposity traits ($P_e > 0.05$) (see supplemental Table 3 for details).

Diabetes incidence was analyzed over the 28 years of follow-up. None of the SNPs offered convincing association with diabetes survival (all P values ≥ 0.05) (see supplementary Table 2). Because a previous study reported that IVS2 + 181G/A was associated with diabetes when an interaction with BMI was added to the model (28), we conducted diabetes incidence analysis with a BMI interaction term included in the model for this SNP, but even with this more refined replication attempt we did not detect a significant association. We also explored the effect of BMI on the association between rs10401670 and diabetes incidence: adding a BMI \times rs10401670 term to the LME model revealed a significant interaction ($P = 0.02$), and the P value for the main effect for rs10401670 reached nominal significance ($P = 0.01$).

The promoter SNP -420C/G (rs1862513) has been investigated by many groups, some examining its association with resistin levels (9,11,13,14,38) and a few with diabetes (29,39) or adiposity (15–18). The analysis of -420C/G (rs1862513) in the Framingham Offspring Study did not show an association with any of the traits measured, including resistin levels. To help attempt to discriminate low power from a true null association, we conducted a meta-analysis of the association of SNP -420C/G (rs1862513) with resistin levels. The details of each population included in the meta-analysis (9,11,13,14,38) and our results are presented in Table 4. The minor C allele seemed to be associated with higher resistin levels; this effect was mainly driven by the largest Japanese study. Heterogeneity was highly significant ($P < 0.0001$). The divergence between studies could be due to differences in ethnic background, age, sex distribution, diabetes status, or other characteristics. When we removed the diabetic subjects from the analysis, heterogeneity was still present.

DISCUSSION

We have demonstrated that circulating resistin levels are associated with SNPs in the 3' region of *RETN* (rs1477341, rs4804765, rs1423096, and rs10401670) in a large, representative community sample. Among the four SNPs that were associated with resistin levels, rs4804765 and rs1423096 showed independent association according to multiple SNP models. One of those, rs10401670, was also associated with mean fasting glucose and FPG at exam 7. Moreover, rs10401670 was nominally associated with diabetes incidence when including a BMI interaction term in the model. No SNP showed significant association with adiposity traits.

Association with resistin and glucose levels. Previous reports of association of SNPs within the *RETN* gene region targeted specific known SNPs and thus achieved

TABLE 4
Results from meta-analysis of the association between SNP -420C/G (rs1862513) and resistin levels*

Study	Ethnic background	Diabetes status	n	Effect (95% CI)	P	%W (random)
Menzaghi et al. 2006	European (Italy)	Nondiabetic	616	-0.15 (-0.52 to 0.22)	0.69	14.09
Cho et al. 2004	Korean	Nondiabetic	173	3.29 (1.31-5.27)	0.001	12.54
		Diabetic	411	2.54 (1.56-3.51)	5.0×10^{-7}	13.73
Yamauchi et al. 2008	Japanese	Nondiabetic	36	10.99 (4.07-17.9)	0.004	5.52
Osawa et al. 2005	Japanese	Diabetic	198	4.88 (3.08-6.68)	2.9×10^{-7}	12.80
Osawa et al. 2007	Japanese	Nondiabetic	1,927	4.97 (4.59-5.34)	2.3×10^{-128}	14.09
		Diabetic	151	5.86 (4.36-7.35)	2.1×10^{-12}	13.19
This study	European (USA)	Nondiabetic	2,020	0.49 (-0.3-1.01)	0.05	14.03
Overall random-effects model				3.51 (1.02-6.00)	0.0009	
Overall heterogeneity					<0.0001	

*Resistin levels in ng/dl. %W, percentage of weight accorded to the study.

only partial coverage; our extensive mapping in and around the *RETN* gene in a large sample has allowed us to reveal novel associations. The four SNPs associated with resistin levels are all located in the 3' region, downstream of *RETN*. SNPs outside of the coding sequence can influence transcription or mRNA stability and thus affect transcript levels. We tried to explore the functional role of those SNPs located in the 3' region of *RETN* by mining publicly available or private genome-wide expression quantitative trait loci datasets, including one obtained from subcutaneous and omental adipose tissue (E.E. Schadt, personal communication). Unfortunately, the fixed marker arrays utilized in these studies do not include our SNPs of interest or any SNPs in moderate to strong LD with them. Thus, specific experiments will need to be carried out to test the functional role of those SNPs on resistin expression and regulation.

Among the SNPs in the 3' region of *RETN*, rs10401670 is associated with both resistin levels and fasting glucose. This supports the notion that resistin is implicated in diabetes pathophysiology. Of note, rs10401670 is located in the second intron of *MCEMP1*, a gene that encodes a 186-amino acid protein with a single transmembrane domain expressed mainly by monocytes and mast cell lines (40). Since resistin is mainly expressed by macrophages that evolve from monocytes in adipose tissue, it would be interesting to know if *MCEMP1* and its product are functionally influenced by rs10401670 or the SNPs in its 5' region and if this protein is involved in resistin and/or glucose metabolism.

Located in the promoter region, rs1862513 (also known as -420C/G) has been investigated by many groups. In a meta-analysis of studies reporting resistin levels and targeting this SNP, we found that the minor allele was significantly associated with higher resistin levels, but a high level of heterogeneity was evident. Removing the diabetic individuals did not eliminate heterogeneity. Residual heterogeneity could be explained by ethnic background. Indeed, the two populations of European descent (ours and a sample from Italy) did not find an association between rs1862513 (-420C/G) and resistin levels (11). The other studies based on Asian populations seemed to show a strong effect, though mainly driven by the largest Japanese study (9). According to the HapMap, the MAF in individuals from European and Japanese descent are comparable (0.33 and 0.35, respectively), which does not explain the difference in the studies included in our meta-analysis. We can hypothesize that there might be gene-gene and/or gene-environment interactions that influ-

ence the two populations differently, but our data does not allow us to make conclusions concerning those possibilities. Also, rs1862513 (-420C/G) might be in LD with a causal SNP in Asians that is not present in individuals of European descent: for example in the HapMap Japanese JPT population, rs1862513 (-420C/G) is in moderate LD ($r^2 = 0.58$) with rs3219175 (also in the promoter region), which is monomorphic in the HapMap CEU population.

Diabetes incidence. We did not find a significant association of any SNP with diabetes incidence, although our sample of incident case subjects was small ($n = 244$). This is concordant with most of the previous literature (9,13,16,23-27). Our best *P* value for association with diabetes survival (age and sex adjusted) was seen with SNP rs3745367 (aka IVS2 + 181G/A; minor allele A increasing the risk hazard ratio to 1.25 [1.00-1.56]), but in the setting of multiple hypothesis, testing this nominal *P* value ($P = 0.05$) cannot convincingly be considered significant. Other reports testing the association between +181G/A and diabetes have been mostly negative (23,25,26); one report showed a positive association in the same direction as our results when including a BMI-gene interaction term in the model (28). Adding a BMI \times genotype interaction term to our diabetes incidence model for SNP rs3745367 did not reveal a significant association. In contrast, adding a BMI \times genotype interaction term to the model with rs10401670 revealed significant interaction, increasing the significance of the (nominal) *P* value of its main effect in predicting diabetes incidence. Since we show that several *RETN* SNPs are associated with circulating resistin levels and that resistin levels are associated with insulin resistance (10), it is possible that a larger sample size might have produced an association with diabetes incidence. Indeed, for a SNP such as rs3745367 of MAF = 0.24 and effect size = 1.25, we had <40% power to detect a significant association with diabetes incidence (see supplemental Table 4). In examining the publicly available meta-analysis of genome-wide association datasets DIAGRAM (<http://www.well.ox.ac.uk/DIAGRAM/meta.html>), only two SNPs in the region of interest were available (rs11883223, rs7408174) and neither one was associated with diabetes. Unfortunately, those two SNPs have very low LD with rs3745367 ($r^2 < 0.10$) or the other SNPs associated with resistin levels in our findings (all $r^2 < 0.02$).

Adiposity traits. Reports of *RETN* associations with BMI or other measures of adiposity in populations of European descent have been inconsistent in the literature (15-20). Some have reported no association (19), while others did so only in subgroup analyses (15-17). The most commonly

investigated variant (−420G allele) has been the subject of several conflicting reports (15–19). Our results are consistent with the notion that *RETN* is not associated with adiposity as assessed by BMI, waist circumference, or body fat composition measured by computed tomography scan. The correlation of adiposity measurements with resistin levels, but not *RETN* genetic variation, suggests that fat accumulation influences resistin levels, but *RETN* variants are not likely to cause weight gain and obesity.

Strengths and limitations. Our study represents a significant advance in its comprehensive coverage of *RETN* and its flanking regions, moderate to high statistical power with a large number of participants in a general community sample including a family-based component, and standardized phenotyping of anthropometric measurements, diabetes, and metabolic traits over 28 years of prospective follow-up. Nevertheless, this study has a few limitations. Power for diabetes incidence was limited (see supplemental Table 4), especially given our expectation of small effect sizes (hazard ratio <1.4). We had adequate power to detect a small proportion of the variance in quantitative traits explained by common SNPs (see supplementary Table 5); for example, we had 85% power to detect 1% of the variance explained (assuming $\alpha = 0.0001$ and an MAF ≥ 0.05), but we may have missed smaller effect sizes in our genotype-phenotype correlations for the resistin levels or glycemic traits. Novel associations need independent replication before we can confidently claim they represent true findings. Currently, studies with large numbers of resistin levels measurements and custom genotyping for comprehensive coverage are uncommon. Also, our findings may need to be refined in populations with LD patterns that differ from those of European descent. Finally, genetic associations do not prove that the SNP is the direct cause of the defect; further fine-mapping and functional studies are needed to identify true causal variants.

Conclusion. We have found that SNPs in the 3' region of *RETN* are associated with circulating resistin levels in the Framingham Offspring Study. One variant (rs10401670) located in the 3' region of *RETN*, but in the second intron of *MCEMP1*, is associated with both resistin levels and fasting glucose. rs10401670 is also nominally associated with diabetes incidence once a putative interaction with BMI is taken into account. Functional studies are needed to investigate the role of *MCEMP1* and to test whether these variants influence *RETN* expression, resistin production, and/or glucose regulation in appropriate tissues. Our new findings need to be replicated in independent data before we can claim that these associations are real: it appears that custom genotyping will be required.

ACKNOWLEDGMENTS

Supported by the National Heart, Lung, and Blood Institute's Framingham Heart Study (contract no. N01-HC-25195); the National Institutes of Health (NIH), National Center for Research Resources (NCRR), General Clinical Research Centers Program (grant no. M01-RR-01066); an American Diabetes Association Career Development Award (to J.B.Me.); a research grant from sanofi-aventis (to J.B.Me.); and the Boston University Linux Cluster for Genetic Analysis (LinGA) funded by the NIH NCRR Shared Instrumentation Grant (1S10RR163736-01A1). M.-F.H. is supported by the Centre de Recherche Medicale de l'Universite de Sherbrooke (CRMUS) and a Canadian

Institute of Health Research (CHIR) Fellowships Health Professional Award. J.B.Me. is also supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant K24 DK080140. J.C.F. is supported by NIH Research Career Award K23 DK65978-05.

J.B.Me. currently has other research grants from Glaxo-SmithKline and sanofi-aventis and serves on consultancy boards for GlaxoSmithKline, sanofi-aventis, Interleukin Genetics, Kalypsis, and Outcomes Sciences. J.C.F. has received consulting honoraria from Publicis Healthcare, Merck, XOMA, and bioStrategies. No other potential conflicts of interest relevant to this article were reported.

We thank all the corresponding authors for their contribution to the meta-analysis data (Claudia Menzaghi, Research Unit of Diabetes and Endocrine Disease, Scientific Institute "Casa Sollievo della Sofferenza," San Giovanni Rotondo, Italy; Haruhiko Osawa, Department of Molecular and Genetic Medicine, Ehime University Graduate School of Medicine, Japan; and Kyong Soo Park, Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea). We also thank Eric E. Schadt for sharing data on genetic determinants of *RETN* expression in adipose tissue.

REFERENCES

1. Scherer PE: Adipose tissue: from lipid storage compartment to endocrine organ. *Diabetes* 55:1537–1545, 2006
2. McTernan PG, Kusminski CM, Kumar S: Resistin. *Curr Opin Lipidol* 17:170–175, 2006
3. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA: The hormone resistin links obesity to diabetes. *Nature* 409:307–312, 2001
4. Fain JN, Cheema PS, Bahouth SW: Resistin release by human adipose tissue explants in primary culture. *Biochem Biophys Res Commun* 300: 674–678, 2003
5. Kaser S, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR: Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. *Biochem Biophys Res Commun* 309:286–290, 2003
6. Patel L, Buckels AC, Kinghorn LJ, Murdock PR, Holbrook JD, Plumpton C, Macphee CH, Smith SA: Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* 300:472–476, 2003
7. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr: Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796–1808, 2003
8. Norata GD, Ongari M, Garlaschelli K, Raselli S, Grigore L, Catapano AL: Plasma resistin levels correlate with determinants of the metabolic syndrome. *Eur J Endocrinol* 156:279–284, 2007
9. Osawa H, Tabara Y, Kawamoto R, Ohashi J, Ochi M, Onuma H, Nishida W, Yamada K, Nakura J, Kohara K, Miki T, Makino H: Plasma resistin, associated with single nucleotide polymorphism-420, is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population. *Diabetes Care* 30:1501–1506, 2007
10. Hivert MF, Sullivan LM, Fox CS, Nathan DM, D'Agostino RB Sr, Wilson PW, Meigs JB: Associations of adiponectin, resistin, and TNF[alpha] with insulin resistance. *J Clin Endocrinol Metab* 93:3165–3172, 2008
11. Menzaghi C, Coco A, Salvemini L, Thompson R, De Cosmo S, Doria A, Trischitta V: Heritability of serum resistin and its genetic correlation with insulin resistance-related features in nondiabetic Caucasians. *J Clin Endocrinol Metab* 91:2792–2795, 2006
12. Azuma K, Oguchi S, Matsubara Y, Mamizuka T, Murata M, Kikuchi H, Watanabe K, Katsukawa F, Yamazaki H, Shimada A, Saruta T: Novel resistin promoter polymorphisms: association with serum resistin level in Japanese obese individuals. *Horm Metab Res* 36:564–570, 2004
13. Cho YM, Youn BS, Chung SS, Kim KW, Lee HK, Yu KY, Park HJ, Shin HD, Park KS: Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. *Diabetologia* 47:559–565, 2004
14. Osawa H, Onuma H, Ochi M, Murakami A, Yamauchi J, Takasuka T, Tanabe F, Shimizu I, Kato K, Nishida W, Yamada K, Tabara Y, Yasukawa M, Fujii Y, Ohashi J, Miki T, Makino H: Resistin SNP-420 determines its

- monocyte mRNA and serum levels inducing type 2 diabetes. *Biochem Biophys Res Commun* 335:596–602, 2005
15. Bouchard L, Weisnagel SJ, Engert JC, Hudson TJ, Bouchard C, Vohl MC, Perusse L: Human resistin gene polymorphism is associated with visceral obesity and fasting and oral glucose stimulated C-peptide in the Quebec Family Study. *J Endocrinol Invest* 27:1003–1009, 2004
16. Engert JC, Vohl M-C, Williams SM, Lepage P, Faith J, Dore C, Renaud Y, Burt NP, Villeneuve A, Hirschhorn JN, Altshuler D, Groop LC, Despres J-P, Gaudet D, Hudson TJ: 5' flanking variants of resistin are associated with obesity. *Diabetes* 51:1629–1634, 2002
17. Mattevi VS, Zembruksi VM, Hutz MH: A resistin gene polymorphism is associated with body mass index in women. *Hum Genet* 115:208–212, 2004
18. Norata GD, Ongari M, Garlaschelli K, Tibolla G, Grigore L, Raselli S, Vettoretti S, Baragetti I, Noto D, Cefalu AB, Buccianti G, Averna M, Catapano AL: Effect of the -420C/G variant of the resistin gene promoter on metabolic syndrome, obesity, myocardial infarction and kidney dysfunction. *J Intern Med* 262:104–112, 2007
19. Smith SR, Bai F, Charbonneau C, Janderova L, Argyropoulos G: A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. *Diabetes* 52:1611–1618, 2003
20. Ukkola O, Kesaniemi YA, Tremblay A, Bouchard C, Kesaniemi YA: Two variants in the resistin gene and the response to long-term overfeeding. *Eur J Clin Nutr* 58:654–659, 2004
21. Xu JY, Sham PC, Xu A, Tso AWK, Wat NMS, Cheng KY, Fong CHY, Janus ED, Lam KSL: Resistin gene polymorphisms and progression of glycaemia in southern Chinese: a 5-year prospective study. *Clin Endocrinol* 66:211–217, 2007
22. Pizzuti A, Argiolas A, Di Paola R, Baratta R, Rauseo A, Bozzali M, Vigneri R, Dallapiccola B, Trischitta V, Frittitta L: An ATG repeat in the 3'-untranslated region of the human resistin gene is associated with a decreased risk of insulin resistance. *J Clin Endocrinol Metab* 87:4403–4406, 2002
23. Conneely KN, Silander K, Scott LJ, Mohlke KL, Lazaridis KN, Valle TT, Tuomilehto J, Bergman RN, Watanabe RM, Buchanan TA, Collins FS, Boehnke M: Variation in the resistin gene is associated with obesity and insulin-related phenotypes in Finnish subjects. *Diabetologia* 47:1782–1788, 2004
24. Gouni-Berthold I, Giannakidou E, Faust M, Kratzsch J, Berthold HK, Krone W: Resistin gene 3'-untranslated region +62G->A polymorphism is associated with hypertension but not diabetes mellitus type 2 in a German population. *J Intern Med* 258:518–526, 2005
25. Kunnari A, Ukkola O, Kesaniemi YA: Resistin polymorphisms are associated with cerebrovascular disease in Finnish type 2 diabetic patients. *Diabet Med* 22:583–589, 2005
26. Ochi M, Osawa H, Onuma H, Murakami A, Nishimiya T, Shimada F, Kato K, Shimizu I, Shishino K, Murase M, Fujii Y, Ohashi J, Makino H: The absence of evidence for major effects of the frequent SNP +299G>A in the resistin gene on susceptibility to insulin resistance syndrome associated with Japanese type 2 diabetes. *Diabetes Res Clin Pract* 61:191–198, 2003
27. Wang H, Chu WS, Hemphill C, Elbein SC: Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. *J Clin Endocrinol Metab* 87:2520–2524, 2002
28. Ma X, Warram JH, Trischitta V, Doria A: Genetic variants at the resistin locus and risk of type 2 diabetes in Caucasians. *J Clin Endocrinol Metab* 87:4407–4410, 2002
29. Ochi M, Osawa H, Hirota Y, Hara K, Tabara Y, Tokuyama Y, Shimizu I, Kanatsuka A, Fujii Y, Ohashi J, Miki T, Nakamura N, Kadowaki T, Itakura M, Kasuga M, Makino H: Frequency of the G/G genotype of resistin single nucleotide polymorphism at -420 appears to be increased in younger-onset type 2 diabetes. *Diabetes* 56:2834–2838, 2007
30. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
31. Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarniecki EM, Schneiderman N, Skyler JS, Marks JB: Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. *Diabetes Res Clin Pract* 47:177–184, 2000
32. Maurovich-Horvat P, Massaro J, Fox CS, Moselewski F, O'Donnell CJ, Hoffmann U: Comparison of anthropometric, area- and volume-based assessment of abdominal subcutaneous and visceral adipose tissue volumes using multi-detector computed tomography. *Int J Obes* 31:500–506, 2007
33. Nathan DM: Labile glycosylated hemoglobin contributes to hemoglobin A1c as measured by liquid chromatography or electrophoresis. *Clin Chem* 27:1261–1263, 1981
34. Almasy L, Blangero J: Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62:1198–1211, 1998
35. Therneau T, Grambsch P: *Modeling Survival Data: Extending the Cox Model*. New York, Springer, 2001
36. Therneau TM, Grambsch PM, Pankratz VS: Penalized survival models and frailty. *J Comput Graph Stat* 12:156–175, 2003
37. DerSimonian R, Laird N: Meta-analysis in clinical trials. *Control Clin Trials* 7:177–188, 1986
38. Yamauchi J, Osawa H, Takasuka T, Ochi M, Murakami A, Nishida W, Onuma H, Takata Y, Tabara Y, Miki T, Ohashi J, Makino H: Serum resistin is reduced by glucose and meal loading in healthy human subjects. *Metabolism* 57:149–156, 2008
39. Osawa H, Yamada K, Onuma H, Murakami A, Ochi M, Kawata H, Nishimiya T, Niiya T, Shimizu I, Nishida W, Hashiramoto M, Kanatsuka A, Fujii Y, Ohashi J, Makino H: The G/G genotype of a resistin single-nucleotide polymorphism at -420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. *Am J Hum Genet* 75:678–686, 2004
40. Li K, Wang S-W, Li Y, Martin RE, Li L, Lu M, Lamhamed-Cherradi S-E, Hu G, Demissie-Sanders S, Zheng J, Chung F, Oates T, Yao Z: Identification and expression of a new type II transmembrane protein in human mast cells. *Genomics* 86:68–75, 2005